AR Biol 14:733-747 1945

THE CHEMISTRY AND METABOLISM OF BACTERIA

By J. Howard Mueller

Department of Bacteriology and Immunology
Harvard Medical School, and School of Public Health
Boston, Massachusetts

It has been necessary to confine the material to be discussed in this review to certain aspects only of the very broad subject. This course has been dictated not only by the limitations of the reviewer but also necessarily by the exigencies of the moment, and by the consideration that certain portions of the subject will undoubtedly be reviewed under other headings. Thus, many of the contributions to bacterial nutrition and to the use of bacteria for purposes of bioassay can appropriately be included in the more general subjects of vitamins, amino acids, etc. The present review is limited to (*a*) a discussion of certain aspects of the chemical composition of the bacterial cell and some of its products, (*b*) that portion of the current work on bacterial nutrition which relates to new and unidentified growth requirements, and (*c*) some phases of chemotherapy.

Complexity of the Bacterial Cell

One of the most remarkable properties shared by many varieties of bacteria is the existence of a multiplicity of immunological types for each species of organism. In a number of instances, for example, the pneumococci in which there are more than thirty such different types, the differences have been found to be concerned with the existence of chemically different polysaccharides which make up the mucoid capsules surrounding these organisms. Moreover, under certain conditions, these bacteria may undergo a degenerative change or "dissociation," in which the capsules and the immunological type specificity disappear. With the loss of the capsule, virulence also disappears. Colonies of such degenerated pneumococci are smaller than those formed by the encapsulated cells and have lost the glossy surface characteristic of the latter. The terms "smooth" (S) and "rough" (R) are commonly used to describe the original form and its variant. In general, rough forms from all types of pneumococci are indistinguishable either culturally or immunologically.

By certain procedures it is possible to reverse this change and to obtain smooth forms from the degenerate rough forms. Under normal

cited by McDermott
re Masked

Obviously

conditions the original type specificity is regained, that is, a rough Type II reverts to a smooth Type II. However, Griffith (1) in 1928 showed that a rough culture obtained from Type II could sometimes be converted to a smooth form with different specificity (e.g., Type III) by injecting mice with a small inoculum of living rough Type II and a large quantity of heat-killed smooth Type III pneumococci. The Type III smooth, virulent organisms so obtained could be maintained indefinitely on suitable media, and it thus appeared that a pneumococcus of one type could be permanently changed to another type by passing through an intermediate rough form. These experiments, adequately confirmed, were extended by Dawson & Sia (2) who devised an *in vitro* method of accomplishing the same result also using living R forms and heat-killed S forms. Still further refinements were introduced by Alloway (3) in which Berkefeld filtered, cell-free extracts of the S cells were substituted for the whole, killed organisms.

Avery, MacLeod & McCarty (4) have now announced the isolation from smooth Type III pneumococci of a substance, provisionally identified as a polymerized desoxyribonucleic acid, which in minute amount possesses the property of inducing the R → S transformation involving change in type specificity. As little as 0.003 μg. in 2.25 cc. of culture fluid was effective. Ultracentrifugation and cataphoresis gave results supporting the conclusion that the nucleic acid polymer was the active substance. Ultraviolet absorption spectra were characteristic of nucleic acid. A tentative estimate of the molecular weight, based on the physical data obtained, led to the figure 500,000. Solutions of the substance were relatively devoid of precipitability by immune sera capable of reacting in high dilution with pneumococcus protein or with Type III specific carbohydrate. In other words, it appears that a polymer of a nucleic acid may be incorporated into a living, degraded cell, and will endow the cell with a property never previously possessed, namely, the ability to produce a capsule composed of a complex polysaccharide entirely different in structure from that produced by the smooth organism from which the degraded form was originally derived. When thus induced the function is permanent, and the nucleic acid itself is also reproduced in cell division. The importance of these observations can scarcely be overestimated and stimulates speculation concerning such matters as the chemical basis for specificity in nucleic acids, and the genetic implications presented by the ability to induce permanent mutation in a cell by the introduction of a chemical substance. Such speculation may well include considerations of the

Avery ↓

relation of this phenomenon to the sequence of events following the introduction of a filterable virus (or a bacteriophage particle) into a susceptible cell. A brief consideration of the facts now accumulated concerning the chemical composition of the viruses may bring this relation into perspective.

It is generally recognized that the most outstanding characteristic of filterable viruses as a group is their failure to multiply outside of the host cell. Beyond this single common property there are wide differences in size and in chemical complexity. From the upper extreme represented by the visible elementary bodies of vaccinia and psittacosis viruses and (for the sake of uniformity of concept) the bacillary forms called rickettsiae, down to the crystalline plant viruses and such agents as poliomyelitis and foot and mouth disease viruses which approach the dimensions of large protein molecules, there are representatives of a wide range of sizes. Similarly, in regard to chemical complexity, while all viruses contain nucleoprotein and the smallest may consist of single molecules of this material alone, the larger ones approach the bacterial cell in complexity, containing lipoid, polysaccharides, and even certain enzymes. Thus as a group viruses may reasonably be considered as living cells deficient in a range of vital functions which must be supplied by a suitable host cell. For example, the larger viruses may be devoid of oxidative mechanisms only, but retain many synthetic capabilities. The latter drop out progressively as size and complexity decrease until nothing remains in the smaller viruses but a large molecule of nucleoprotein multiplying as a result of the combined vital processes of the host cell.

Considerable definite information relating to the chemical composition of the largest and the smallest of the viruses has been available for some time. Information has now been provided by Taylor and others about viruses of intermediate size (5, 6, 7), namely influenza viruses A and B and swine influenza virus. Lipoid components varied from 21 to 24 per cent among the three, and included neutral fat, phospholipid, and cholesterol. The remainder consisted of polysaccharide, protein, and desoxyribonucleic acid. Even these viruses of intermediate size, therefore, are still relatively complex entities. It would be interesting to know whether they still possess certain enzymes such as phosphatase which has been found to be present in vaccinia virus (8) though absent from small viruses such as the polyhedral virus of *Lymantria monacha* L. (9), and tobacco mosaic and bushy stunt viruses (10). Williams, Schlenk & Eppright (11) re-

— Dubos' Book. Not cited

ported the absence of the various components of the B vitamin complex from tobacco mosaic, tobacco necrosis, and bushy stunt viruses and from a strain of influenza A virus. They believe such absence carries implications of the inanimate nature of viruses. In the light of the above discussion it seems probable that the cellular functions dependent upon these readily extractible substances may well be missing from even the largest viruses, which may yet retain so complex a make up and properties so varied as to bring their inanimate nature into considerable question.

The one component common to all viruses, therefore, is nucleoprotein, while in the case of the substance inducing variation in the pneumococcus, nucleic acid alone is involved. In the latter case a synthetic and purposeful change is brought about, i.e., the ability to produce a polysaccharide capsule rendering the cell resistant to certain destructive influences. On the other hand, so far as is known, viruses lead to degenerative changes in the host cell and eventually to its death. Conceivably, therefore, the crucial difference between the transplanted "gene" and the smallest virus may lie in the specificity of the protein which is present in the latter, or to which the former probably attaches itself in the cell. In the one case the protein heterologous to the cell, renders the particle a completely foreign parasitic molecule, which eventually becomes injurious as it accumulates. In the other instance, the cell's own protein combines with the new nucleic acid, and the resulting homologous nucleoprotein takes its place in the normal economy of the cell and confers a new property upon it. In any case, these considerations again indicate the enormous complexity of cellular and bacterial protoplasm, and emphasize our complete ignorance of the many and varied metabolic processes which proceed inside the cell wall.

As further illustrations of the great complexity of the bacterial cell a few instances from the contributions of the current year may be mentioned, selected primarily because of some bearing on medical bacteriology.

Stockinger, Ackerman & Carpenter, for example, reported extensive studies of the composition of the gonococcus (12) and its products (13). They described two nucleoprotein fractions obtained by extraction of mass cultures. One of these contained considerable combined lipoid. Carbohydrate was found in three different forms, but a polysaccharide having type specific properties was not detected. A variety of lipoidal constituents were present, among which a lecithin,

a cephalin, and sphingomyelin were identified. From broth in which the gonococcus had been grown, they isolated a protein, believed to be a degradation product of the cellular nucleoprotein. It was moderately toxic to animals and possessed immunological specificity as measured by complement fixation. Boor & Miller (14) prepared carbohydrate-lipid complexes from a number of strains of gonococcus and meningococcus. These "glucolipids" were also moderately toxic for animals and were antigenic in rabbits. Sera so produced precipitated both the glucolipid itself and the carbohydrate component separated by acid hydrolysis. These complexes, therefore, have properties similar to compounds of the same sort prepared earlier by Boivin & Mesrobeanu (15, 16) from a variety of gram negative bacilli.

Kabat, Kaiser & Sikorski (17) have prepared a polysaccharide from Type I meningococci which is electrophoretically homogeneous, and which is type-specific. It is weakly but definitely antigenic in man. Direct comparison with an earlier product obtained by Scherp & Rake (18) indicates that the avoidance of acid or alkali during the preparation of the more recent material has resulted in a somewhat purer and more nearly natural antigenic substance.

An antigenic capsular polysaccharide has been obtained from *Cl. perfringens* by Svec & McCoy (31). It appears to be common to most members of the *perfringens* group, regardless of their toxigenic properties.

The tremendous amount of investigation on the chemistry of tuberculin carried out in the last two decades has been reviewed during the year by Seibert (19), particularly in regard to carbohydrates, nucleic acid, and protein. Corper & Cohn (20) have compared the tuberculoprotein obtained from autolyzed tubercle bacilli with that present in culture filtrates, and found similar properties, together with greater purity, in the former. Chargaff & Moore (32) described the isolation of glycogen from tubercle bacilli. The molecular weight of the material was shown to be of the order of 12 to 13 million. Anderson and his collaborators (21, 22, 23) have added to an already extensive investigation of the lipids of the tubercle bacillus, and carried out similar studies (24, 25) with *Phytomonas tumefaciens,* incited by the possibility that some of its lipid material may be responsible for the ability of the organism to induce plant galls. An unidentified fat acid with the formula $C_{20}H_{40}O_2$ was isolated. It is believed to possess a branched chain.

The capsular material of certain hemolytic streptococci, identified